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Synchronous production of protoperithecia

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Abstract
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Ho C.C. Synchronous production of protoperithecia. The following method produces large quantities of protoperithecia which can be harvested reasonably free from vegetative hyphae and conidio. It can be used to study changes in enzyme levels &ring the differentiation of this organelle.

Two drops of conidial suspension from a Pasteur pipette (conidial concentration = all conidia produced by a four-day-old culture grown in 1 ml slope and suspended in 2 ml of water) are inoculated into 40 ml of modified liquid medium of Westergaard and Mitchell (1947 Am. J. Botany 34:573) containing the trace elements of Vogel's medium N (1964 Amer. Nat. 98:435), low sulfur (7.85 mg $MgSO_4.7H_2O$ and 0.4 gm $MgCl_2.6H_2O$ instead of the usual 0.5 gm $MgSO_4$ per liter), high bictin (250 μg instead of 5 μg per liter) and 2% sucrose. The pH is adjusted to 6.7 with sodium hydroxide. The high biotin concentration may not be essential

Ten ml of this liquid medium is poured on top of a thick layer of solid 2% water agar (Bacto, Difco) contained in a tall Pyrex petri dish (100 mm dia. x 80 mm ht.). The dish is tilted to one side so that three-quarters of the agar surface is above the liquid and is incubated in the dark at 25°C. After 3 days, the remaining liquid medium is decanted, which at the same time washes away the conidia sticking on the walls of the dish. During harvesting, the mycelia grown in liquid do not stick to the agar surface and so can be lifted out. The protoperithecia on the dried agar surface are scraped off with a small blade, with most of the vegetative hyphae sticking on the agar. The harvested structures are squeezed free of liquid and then stored at -15°C before enzyme assays. The conidia are generally found on the vertical walls of the dish and are washed away prior to harvesting.

For wild type strains, about 40 protoperithecia per cm2 are formed on the dried agar surface from the second to the third day There is, however, further increase of weight for bath protoperithecia and mycelia grown previously on liquid medium after the (Table 1). There is, however, further increase of weight for both protoperithecia and mycelia grown previously on liquid medium after the third day. Protoperithecia also develop on mycelia grown in liquid medium. Most of them are formed from the fourth to the fifth day. This method allows the production of large numbers of protoperithecia of the sane age on the dried agar surface, which can then be harvested relatively free of mycelia. The protoperithecia developed under these conditions can be mated to form perithecia with normal ascospores. Pure prithecia con also be collected, as was done for protoperithecia.

[N.B. Table 1 is on p. 18]

A protoperithecia-less (ff-1) mutant (Tan and Ha 1970 Mol. Gen. Genet. 107: 158) also doer not develop protoperithecio under these conditions. Several other mutants defective in protoperithecial development hove also been cultured. For several of these, the aerial hyphae growing on the vertical walls of the dish are shorter than those of wild type. (This work was carried out under a Gosney Fellowship at the laboratory of N. H. Horowitz, Division of Biology, California Institute of Technology) - - - Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.